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3. Full name, address and postcode of the or of each patent applicant *(underline all surnames)*

PPL Therapeutics (Scotland) Limited
Roslin
Midlothian
Edinburgh EH25 9PP

Patents ADP number *(if you know it)* 6715858002

If the applicant is a corporate body, give the country/state of its incorporation GB

4. Title of the invention

PURIFICATION

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Signature: Kilburn & Strode

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PURIFICATION

This disclosure is concerned generally with protein purification from milk and specifically with the purification of human fibrinogen from the milk of transgenic non-human animals.

Fibrinogen, the main structural protein in the blood responsible for the formation of clots exists as a dimer of three polypeptide chains; the Aα (66.5kD), Bβ (52kD) and γ (46.5kD) are linked through 29 disulphide bonds. The addition of asparagine-linked carbohydrates to the Bβ and γ chains results in a molecule with a molecular weight of 340kD. Fibrinogen has a trinodal structure, a central nodule, termed the E domain, contains the amino-termini of all 6 chains including the fibrinopeptides (Fp) while the two distal nodules termed D domains contain the carboxy-termini of the Aα, Bβ and γ chains. Fibrinogen is proteolytically cleaved at the amino terminus of the Aα and Bβ chains releasing fibrinopeptides A and B (FpA & FpB) and converted to fibrin monomer by thrombin, a serine protease that is converted from its inactive form by Factor Xa. The resultant fibrin monomers non-covalently assemble into protofibrils by DE contacts on neighbouring fibrin molecules. This imposes a half staggered overlap mode of building the fibrin polymer chain. Contacts are also established lengthwise between adjacent D domains (DD contacts) leading to lateral aggregation. Another serine protease, Factor XIII is proteolytically cleaved by thrombin in the presence of Ca^{2+} into an activated form. This activated Factor XIII (Factor XIIIa) catalyses crosslinking of the polymerised fibrin by creating isopeptide bonds between lysine and glutamine side chains. The first glutamyl-lysyl bonds to form are on the C-terminal of the γ chains producing D-D crosslinks. Subsequently, multiple crosslinks form between adjacent Aα chains, the process of crosslinking imparts on the clot both biological stability (resistance to fibrinolysis) and mechanical stability [Sienbenlist and Mosesson, Progressive Cross-Linking of Fibrin

γ chains Increases Resistance to Fibrinolysis, Journal of Biological Chemistry, 269: 28414-2841, 1994].

The coagulation process can readily be engineered into a self sustained adhesive system *in vitro* by having the fibrinogen and Factor XIII as one component and thrombin and Ca^{2+} as the second component which catalysis the polymerisation process. These adhesion systems, know in the art as "Fibrin Sealents" or "Fibrin Tissue Adhesives" have found numerous application in surgical procedures and as delivery devices for a range of pharmaceutically active compounds [Sierra, Fibrin Sealent Adhesive Systems: A Review of Their Chemistry, material Properties and Clinical Applications, Journal of Biomaterials Applications, 7:309-352, 1993].

It has been estimated that the annual US clinical need for fibrin sealents is greatly in excess of the 300Kg/year that can be harvested using the current cryoprecipitation methods used by plasma fractionaters. Alternative sources of fibrinogen, by far the major component in fibrin sealent, have therefore been explored with recombinant sources being favored [Butler et al., Current Progress in the Production of Recombinant Human Fibrinogen in the Milk of Transgenic animals, Thrombosis and Haemostasis, 78: 537-542, 1997]. It has been shown that mammals are capable of producing transgenic human fibrinogen at levels of up to 5.0g/L in their milk making this a commercially viable method for the production of human fibrinogen [Prunkard et al., High-level expression of recombinant human fibrinogen in the milk of transgenic mice, Nature Biotechnology, 14:867-871, 1996; Cottingham et al., Human fibrinogen from the milk of transgenic sheep. In: Tissue Sealents: Current Practice, Future Uses. Cambridge Institute, Newton Upper Falls, MA, March 30 April 2 1996 (abstract)].

Differences have been identified between recombinant human fibrinogen and

fibrinogen which has been purified from human plasma. Fibrinogen which has been purified from human plasma has two alternately spliced gamma chains (γ and γ'). In contrast, recombinant human fibrinogen only has the major form γ. Further, the glycosylation of the beta and gamma chains (there is no N-linked glycosylation of the alpha chain) of recombinant human fibrinogen differs slightly from that on plasma derived fibrinogen, but is similar to the glycosylation found on other proteins expressed in the milk of transgenic animals. In addition, the Ser3 of the alpha chain of recombinant human fibrinogen is more highly phosphorylated than Ser3 of the alpha chain of plasma derived fibrinogen, although the difference in phosphorylation does not result in functional differences. Also, there are detectable differences in heterogeneity caused by C-terminal proteolysis of a number of highly protease–sensitive sites on the alpha chain. Differences of a similar magnitude are also observed between plasma-derived fibrinogen from different sources.

Milk is well known to contain a number of serine proteases; of these, the alkaline protease plasmin, which occurs in milk together with its inactive zymogen plasminogen, is the most significant protease contributing to proteolytic activity. Plasmin(ogen) concentration varies with health status of the animal e.g. mastitic animals exhibit increased proteolytic activity. Also influencing the proteolytic activity of milk is stage in lactation i.e. late lactation is associated with higher concentrations of plasmin [Politis and Ng Kwai Hang, Environmental Factors Affecting Plasmin Activity in Milk, Journal of Dairy Science, 72:1713-1718, 1989]. In milk, plasmin(ogen) is associated predominantly with the casein micelles, although it can also be found to a lesser extent in whey [Politis et al., Distribution of Plasminogen and Plasmin in Fractions of Bovine Milk, Journal of Dairy Science, 75:1402-1410, 1992].

Plasmin is the serine protease that is predominantly responsible for the dissolution of fibrin clots *in vivo* and its presence is essential for haemostasis. It is very probable that any fibrinogen degradation product in milk is as a result of the action of milk proteases. Therefore, the presence of plasmin or other proteases in milk can be detrimental to the quality of fibrinogen that is produced by the lactating transgenic animal if steps are not taken to minimize their effect. Of equal importance is the removal of any fibrinogen degradation products that may result from the action of plasmin or other milk proteases. The use of protease inhibitors to minimize proteolysis is well established in the art and usually involves the addition of a cocktail of inhibitors of varying specificity. With transgenic animals the possibility of proteolytic damage to the recombinant protein has been realized and suggestions have been put forward to limit degradation (Wilkins and Velander, Isolation of Recombinant Proteins from Milk, Journal of Cellular Biochemistry, 49:33-338, 1992; Velander et al., PCT WO 95/22249). However, increasingly effective methods are constantly a desideratum.

In the purification of proteins from milk, one requirement is the separation of the desired protein from contaminating casein micelles. For the isolation of transgenic proteins such as AAT, the first step is precipitation with PEG or other agent, such as ammonium sulphate. This does not precipitate AAT, but precipitates casein and is therefore a good way of removing casein from the AAT. However, when this teaching was applied to transgenic fibrinogen in milk, it was found that not only did the casein precipitate, but that the fibrinogen precipitated with it. This was clearly not a good step for removing casein from fibrinogen. Further, the fibrinogen in the casein/fibrinogen precipitate was unstable and was very quickly proteolytically damaged, probably due to the co-precipitation with protease enzymes. The problem was thus how to separate casein from fibrinogen-like proteins in a milk sample or

fraction thereof. The separation of plasmin(ogen) from casein micelles can be accomplished by incubation with agents such as 6-aminohexanoic acid (ε-aminocaproic acid, εACA). However, 6-aminohexanoic acid also increases the activation of plasminogen to plasmin which may accelerate proteolysis of any susceptible desired protein. Furthermore, the separation of plasmin (or plasminogen) from casein micelles does not assist in the separation of casein micelles from fibrinogen-like proteins.

Accordingly, there remains a need to separate desired proteins from casein micelles without accelerating proteolysis of the desired protein.

The invention provides an efficient and effective method whereby a protein produced in the milk of transgenic animals is recovered and purified.

Accordingly, the present invention provides a method for the part purification of a desired protein from milk, the method comprising the transfer of protease enzyme which is present in the milk, into the whey phase with the removal or partition of the desired protein into another phase of the milk.

In the present text, the term "part purification" means purification to a level of from 50% free from other contaminants, preferably 60, 70, 80, 90% free from other contaminants. Preferably the recovery rates are in the range 50% to about 80%, more preferably in the range 65% to 85%.

The desired proteins according to the present invention are any of those which may be produced in milk, including naturally produced milk proteins and transgenic proteins. Preferred proteins according to the present invention are those having fibrinogen-like characteristics which result in co-precipitation with casein in the

presence of PEG or ammonium sulphate. Such proteins include, but are not limited to; fibrinogen, collagen, fibronectin, Factor VIII and alpha-2-macroglobulin.

The present invention is preferably in relation to the isolation of transgenic proteins from milk, that is proteins produced as a result of transgenic manipulation of an animal. This accordingly allows for the isolation of proteins, such as fibrinogen, collagen, fibronectin, Factor VIII and alpha-2-macroglobulin from animal milk which does not normally contain such proteins. The present invention is useful for the production and isolation of individual proteins *per se*, or proteins which have been altered in some way to facilitate transgenic expression, such as by fusion to other proteins.

As used herein, the term "fibrinogen" refers to the main structural protein responsible for the formation of clots and includes the whole glycoprotein form of fibrinogen as well as other related fibrinogen species, including truncated fibrinogen, amino acid sequence variants (muteins or polymorphic variants) of fibrinogen a fibrinogen species which comprises additional residues and any naturally occurring variants thereof. The same variations as described above also apply to other fibrinogen-like proteins which can be isolated from milk according to the present invention.

As use herein, "milk" is understood to be the fluid secreted from the memory glands in animals. Milk according to present invention includes whole milk, skimmed milk, milk fraction, colosteral milk or a milk-derived fluid where the desired protein was originally produced in milk.

The present invention enables the part purification of the desired protein by transferring protease enzymes present in the milk away from the phase into

which the desired protein is obtained. The protease enzyme is transferred into the whey phase ((whey phase being the phase/portion/fraction of milk which contains predominantly non-casein proteins) with the removal or partition of the desired protein into another phase of the milk. The removal or partition of the desired protein may be simultaneous to the transfer of the protease enzyme in the whey phase. Alternatively, it is possible to have a two-step process, whereby the protease enzyme is transferred first to the whey under conditions which retard proteolytic damage to the desired protein, followed by the removal or partition of the desired protein. Such conditions can be constructed by using protease inhibitors or low temperature.

The transfer of protease enzyme into the whey phase predominately relates to the transfer of plasmin and/or plasminogen. Other milk proteases, such as serine proteases (alkaline or acid) may also be transferred.

The desired protein is recovered from the milk by the use of precipitation techniques well known to those in the art, such as by the use of protein precipitation agents including, but not exclusively, PEG, sodium sulphate, ammonium sulphate, glycine or temperature. The precipitation is preferably carried out with generally low concentrations of the chemical precipitation agents (e.g. 5-20% w/v sodium and ammonium sulphate, 5-20% w/v glycine or β-alanine; 2-15% PEG) as this reduces co-precipitation of whey proteins.

The transfer of the protease enzyme into the whey phase of the milk is preferably by the presence of lysine or lysine analogue such as ε-aminocaproic acid or other basic amino acids, such as arginine or histidine. The concentration of lysine or a lysine analogue according to the invention would depend on a number of factors such as the type of milk from which the desired protein is

being purified, the amount of the desired protein present and the manner of removal or partition of the desired protein from the way phase of the milk. Concentrations typically range from 1mM-2M, preferably 10-200mM.

Most preferably, the method of the first aspect of the invention is repeated at least once, and up to approximately four times. This repetition can greatly increase the purity of the desired proteins in particular in respect of contaminating micelles.

The method according to the first aspect of the invention increases the stability of the part purified desired protein toward proteolysis, especially when the desired protein is a transgenic protein.

A second aspect of the invention provides a method for the part purification of the desired protein from milk, the method comprising precipitation of the desired protein in the presence of lysine or a lysine analogue.

All relevant preferred features of the first aspect of the invention, also apply to the second.

The milk from which the desired protein is to be part purified is preferably derived from animals which can be "farmed" in order to produce sufficient quantities of milk from which to obtain pharmaceutical proteins and include sheep, cows, goats, rabbits, camel, water buffalo, pig or horse. Such animals may clearly be transgenically modified animals. Preferably, although not exclusively, the transgenic protein is bovine or human derived. Human derived proteins are preferable as these, when isolated and purified for pharmaceutical

use from the milk of a transgenic animal are less likely to cause an unwanted immunological reaction when administered to a human in need thereof for medicinal purposes. The present invention does not relate to transgenically modified humans.

The plasminogen activation system in milk has been a focus of interest for a number of years. It is generally accepted that milk contains the primary enzymes responsible for fibrinolysis *in vivo* e.g. plasminogen activator (both tissue type, tPA and urokinase type, uPA], plasminogen and plasmin. The action of proteolysis is often observed during storage of milk or milk products where casein appears to be the milk protein most susceptible to degradation. It was soon illustrated that in milk, plasminogen activators, plasminogen and plasmin were associated mainly with the casein micelles and not in the whey (or serum) phase. The mechanism by which these molecules associate with casein has not been categorically determined but it is probable that as these molecules contain Kringle domians (structured polypeptide chains with an affinity for basic amino acids) these domains probably mediate their interaction with casein. Heegaard et al., 1997 [Plasminogen Activation System in Human Milk, Journal of Paediatric Gastroenterology and Nutrition, 25: 159-166] have shown that casein immobilised on Sepharose is capable of binding tPA and when casein is present, the tPA catalysed conversion of plasminogen to plasmin is accelerated. This seems to suggest that the juxtaposition of casein, plasminogen and tPA results in enhanced plasminogen activation. The mechanism of enhanced activation is not clear but may be due to plasminogen undergoing a conformational change on binding to casein resulting in a molecule more readily activated with tPA [Markus et al., Casein, A Powerful Enhancer of the rate of Plasminogen Activation, Fibrinolysis 7: 229-236]. It is therefore

apparent that an agent (such as Lysine or Lysine analogue) added to milk in sufficient concentration will dissociate tPA and plasmin(ogen) from casein transferring them to the whey phase. The consequences of this are that active plasmin and plasminogen are then present in the same phase as the transgenic protein. In terms of fibrinogen, as discussed above, the result of this is that proteolysis, especially of the Aα chain will occur. It is known in the art that εACA is relatively ineffective at inhibiting primary fibrinolysis i.e Fragment X (F3) formation from fibrinogen or fibrin and it has been postulated that initial degradation of fibrin may occur independent of noncovalent plasmin-fibrin interaction (which is mediated through kringle domains on plasminogen binding to basic amino acids in the fibrinogen Aα chain), unlike the later steps which result in the formation of fragments Y, D and E. Indeed it has been shown [Francis et al., Structural and Chromatographic Heterogeneity of Normal Plasma Fibrinogen associated with the Presence of Three γ-chain types with Distinct Molecular Weights, Biochimica et Biophysica Acta, 744: 155-164] that Aα chain proteolysis in commercial fibrinogen preparations proceeds during chromatographic separation into fibrinogen sub-families even with the inclusion of 20mM ε-Aminocaproic acid and Aprotinin (a potent protease inhibitor) at 10 Kallikrein units/ml. It is therefore apparent that addition of ε-Aminocaproic acid during the purification of human fibrinogen from milk would have no beneficial, and even negative effects.

Paradoxically we have discovered that ε-aminocaproic acid is a useful aid in preventing degradation of fibrinogen during its purification from milk if it is included during a stage which partitions the fibrinogen, such as a precipitation stage. The similarity between fibrinogen and casein in terms of susceptibility to precipitation; a technique widely used in the purification of fibrinogen from

plasma and cryoprecipitate [e.g. Schwarz et al., US Patents 4,362,567; 4,377,572 & 4,414,976], and in the separation of casein from milk [Swaisgood, Developments in dairy Chemistry – 1: Chemistry of Milk Protein, Applied Science Publishers, NY, 1982] leads to the co-precipitation of at least part of the casein fraction when precipitating fibrinogen from milk using precipitating agents well known to those in the art (e.g. but not exclusively Zinc, Copper, sodium and ammonium salts, amino acids (e.g. glycine, β alanine), alcohol (e.g.ethanol) and polymers (e.g. polyethylene glycol, dextran or hydroxyethyl starch. Even by adding these precipitants at relatively low concentrations (e.g 5-20% w/v sodium and ammonium sulphate, 5-20% w/v glycine or β-alanine; 2-15% PEG) sufficient to precipitate fibrinogen or a majority fraction of it also co-precipitates a fraction of the casein phase including some whey proteins. This can be reduced if the precipitation is carried out more than once. The inclusion of ε-aminocaproic acid or a similar analogue of lysine during the precipitation stage (at a concentration of 10-200mM) results in the dissociation of kringle containing proteins from casein and fibrinogen and maintains them in the solution phase while the fibrinogen is precipitated. The method of protection of the fibrinogen is therefore one of exclusion. The precipitated fibrinogen can then be reconstituted in a suitable buffer and is not only significantly less susceptible to proteolysis but also significantly more pure. Such a technique would work equally well if temperature is used as a method of precipitation. The added advantage of this invention is that not only is the ε-aminocaproic acid preventing proteolytic damage to the fibrinogen, it does not contaminate the precipitated fibrinogen as it remains in the solution phase.

A third aspect of the present invention provides a transgenic protein, at least partly purified, having improved stability, in particular in respect of proteolysis.

All preferred features of the first and second aspects of the invention also apply to the third, even though the transgenic protein of the third aspect may not necessarily be required to be produced according to the method of the first or second aspect.

A fourth aspect of the invention provides a protein obtainable by a method according to the first or second aspects of the invention. All preferred features of the first and second aspects of the invention, also apply to the fourth.

A fifth aspect of the invention provides for the use of lysine or a lysine analogue in the purification of a transgenic protein from milk. The use of the lysine or lysine analogue according to the fifth aspect of the invention is preferably in combination with the precipitation of the transgenic protein.

All preferred features, where relevant, according to the first to fourth aspects of the invention, will also apply to the fifth.

A sixth aspect of the invention provides a fibrin adhesive or sealent containing fibrinogen according to the third or fourth aspects of the invention. The fibrin adhesive or sealent according to the sixth aspect of the invention are, in all aspects, with the exception of the particular fibrinogen used, well known and standard in the art [Sierra, Fibrin Sealent Adhesive Systems: A Review of Their Chemistry, Material Properties and Clinical Applications, Journal of Biomaterials Applications, 7:309-352, 1993; Martinowitz and Spotnitz, Fibrin Tissue Adhesives, Thrombosis and Haemostasis, 78:661-666, 1997; Radosevich *et al.*, Fibrin Sealent:Scientific Rationale, Production Methods, Properties and Current Clinical Use, Vox Sanguinis, 72:133-143, 1997].

As used herein, the term "fibrin adhesive" or "fibrin sealent" describes a substance containing fibrinogen which is capable of forming a biodegradable adhesive or seal by the formation of polymerised fibrin. Such adhesive/sealent systems are alternatively called "fibrin tissue adhesives" or "fibrin tissue glues". The adhesive or seal may act as, *inter alia* a hemostatic agent, a barrier to fluid, a space-filling matrix or a drug-delivery agent. Particular use may be found in neurosurgery, opthalmic, orthopedic or cardiothoracic surgery, skin grafting and various other types of surgery.

Other than fibrinogen, the fibrin adhesive or sealent may contain substances which encourage the formation of the fibrin adhesive/seal, such as thrombin, Ca^{++} and Factor XIII (which in this text also includes reference to Factor XIIIa). While it is recognised that thrombin would be the preferred enzyme with which to incorporate into any system whereby the formation of a fibrin clot is desired, it is appreciated that there are other enzymes capable of proteolytically cleaving fibrinogen resulting in the formation of a fibrin clot. An example of this would be the snake venom enzyme Batroxobin [Weisel and Cederholm-Williams, Fibrinogen and Fibrin: Characterization, Processing and Applications, *Handbook of Biodegradable Polymers* (Series: Drug targeting and Delivery) 7:347-365, 1997]. Other components such as albumin, fibronectin, solubilisers, bulking agents and/or suitable carriers or diluents may also be included if desired.

One advantage of fibrin sealent as a biodegradable polymer is that there are natural mechanisms in the body for the efficient removal of clots and thus the fibrin sealent may be a temporary plug for hemostasis or wound healing. Various proteolytic enzymes and cells can dissolve fibrin depending on the circumstances, but the most

specific mechanism involves the fibrinolytic system. The dissolution of fibrin clots under physiological conditions involves the binding of circulating plasminogen to fibrin, and the activation of plasminogen to the active protease, plasmin, by plasminogen activators which may also be, also bound to fibrin. Plasmin then cleaves fibrin at specific sites.

Depending on the situation, it may be advantageous to let the natural process of fibrin breakdown take place after applying a fibrin adhesive or sealent to a site. Indeed, this breakdown may be encouraged, for example, by the inclusion of plasminogen. Alternatively, in some situations it may be advantageous to delay the process by including antifibrinolytic compounds which can, for example, block the conversion of plasminogen to plasmin or directly bind to the active site of plasmin to inhibit fibrinolysis. Such antifibrinolytics include α_2-macroglobulin, which is a primary physiological inhibitor of plasmin; aprotinin; α_2-antiplasmin; and ε-aminocaproic acid.

The fibrin/sealent may comprise two components, one component containing fibrinogen and Factor XIII (and/or Factor XIIIa) and the other component containing thrombin and Ca^{++}. Other substances as described above may be included in one or both of the components if desired.

All the preferred features of the first to sixth aspects of the invention, also apply to the seventh.

All relevant preferred features of the first to fifth aspects of the invention, also apply to the sixth.

A seventh aspect of the invention provides a kit for a fibrin adhesive or sealent comprising fibrinogen according to the third or fourth aspects of the invention, and instructions for use or, may comprise fibrinogen according to the third or fourth aspect of the invention in combination with (but not necessarily mixed with) one or more of: Factor XIII, Factor XIIIa, thrombin or Ca^{++}. Furthermore, the kit may comprise two components: fibrinogen with (but not necessarily mixed with) Factor XIII (and/or Factor XIIIa) and thrombin with (but not necessarily mixed with) Ca^{++}.

All relevant preferred features of the first to sixth aspects of the invention also apply to the seventh.

An eighth aspect of the invention provides a method for producing a fibrin adhesive or sealent according to the sixth aspect of the invention, comprising admixing fibrinogen with thrombin or any other enzyme which is capable of proteolytically modifying fibrinogen and causing it to clot. Factor XIII (and/or Factor XIIIa) and Ca^{2+} may also be mixed with the fibrinogen and thrombin (or other suitable enzyme) in this aspect of the invention.

The method of admixing fibrinogen and thrombin may involve squirting or spraying the components simultaneously or sequentially to the repair site with a syringe or a related device. The mixing may result from two syringes held together along their barrels and at the plunders with two components mixed either after exiting the needles or in the hub just prior to exiting. Other devices may be used to produce an aerosol or to spray in a variable pattern, depending on the application.

Although various derivatives of fibrinogen have been used in clinical applications for some time, there are several safety issues involved in the clinical use of fibrinogen such as concern over viral contamination, especially with products containing fibrinogen or components prepared from human blood especially pooled human blood. Although improvements in viral cleansing techniques for blood products have been made since the fear of transmission of pathogenic viruses was brought to the surface, so that the risk of disease transmission has been greatly reduced, the risk has not been totally eliminated. The present invention, which relates to fibrinogen obtained from milk, can be substantially free from such a concerns.

A ninth aspect of the invention provides fibrinogen, according to the second or third aspect of the invention, for use in medicine. Preferably the fibrinogen is used in human medicine, However, it may also be used in veterinary medicine such as for horses, pigs, sheep, cattle, mice and rats as well as for domestic pets such as dogs and cats.

While the main use of fibrinogen is thought to be for the preparation of adhesive or sealing agents as hereinbefore described, fibrinogen has other applications in the field of medicine, for example as a coating for polymeric articles as disclosed in US Patent No 5,272,074. A particular use of lyophilised fibrinogen of the present invention is within or part of a gauze or bandage (preferably made from polylactic acid compounds used in surgical stitches). Such a wound dressing can be supplied (also incorporating the other components required for the formation of a clot (described above), optionally in a package or kit form, for application direct to the skin or to an internal organ. All preferred features of previously discussed aspects, also apply to the ninth.

A tenth aspect of the invention provides a fibrin adhesive or sealent, according to the sixth or seventh aspect of the invention, for use in medicine.

The use in medicine may be any of those described herein. All preferred features of aspects one to nine, also apply to the tenth.

An eleventh aspect of the present invention provides a method of surgery or therapy comprising placing fibrinogen according to the third or fourth aspect of the invention, on or within a animal or a body part of an animal. Preferably the animal is a human. The fibrinogen may be mixed with one or more of thrombin, Factor XIII, Factor XIIIa or Ca^{2+} separately, sequentially or simultaneously with the fibrinogen. The fibrinogen may thus be in the form of a sealent according to the sixth aspect of the invention. The fibrinogen may be applied by squirting using a syringe or a related device. It may be applied very precisely in a localised area or broadly over a wide area to any tissue. Preferred features of aspects one to ten also apply to the eleventh.

A twelfth aspect of the invention provides the use of fibrinogen, according to the second or third aspects of the invention in the manufacture of a fibrin adhesive or sealent.

The following non limiting example help to illustrate this invention.

Example 1

Milk from a transgenic ewe was thawed from a frozen state in a water bath at 37°C and then delipidated by low speed centrifugation (2000rpm) for 10 minutes. The skimmed milk was than aliquoted into 2 x 40ml fractions and processed as follows. To one of the fractions was added 40ml of 27.6% (w/v) ammonium sulphate in 25mM citrate, 100mM εACA, pH 8.0. The tube was mixed for 20 minutes at room temperature followed by high speed centifugation in a Beckman J2-21 centrifuge (15°C). The supernatant generated was removed and the pellet dissolved in 25mM citrate, pH 8.0. Once dissolved up to 40ml, the precipitation and resolubilisation was repeated as above. A final precipitation and resolubilisation step was then carried out, essentially as above except that εACA was omitted from the salt solution. The same process as above was then repeated on the second 40ml aliquot of skimmed milk except that εACA was not used.

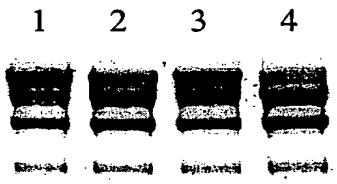

Figure 1. SDS-PAGE analysis of part-purified fibrinogen in the absence or presence of εACA.

The Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE, 8-16%, Novex) shown as Figure 1 illustrates the stability of part purified fibrinogen. Lane 1 represents fibrinogen part purified in the presence of εACA and stored at 4°C overnight. It can be seen that the fibrinogen is

predominantly F1 : F2. The degradation product, Fragment X (F3) is also present as a faster migrating band under the F1 : F2 bands. Lane 2 represents material stored at 4°C purified as in Lane 1 except that εACA was absent during the precipitation stage. From Lane 1 and Lane 2, it is evident that some F1 fibrinogen has been proteolytically cleaved even during storage at 4°C. Lane 3 represents material as in lane 1 except that storage was at 18°C overnight. As can be seen this material appears to be more stable than that shown in Lane 2 and in fact is very similar to that shown in Lane 1. Lane 4 represents material purified in the absence of εACA after overnight storage at 18°C. It is evident that this material has been severely damaged and is almost lacking in F1 fibrinogen. This example serves to illustrate that fibrinogen, part purified from milk by precipitation, is unstable to milk protease action. This protease action may be diminished by incubation at 4°C but is abolished if the precipitation is carried out in the presence of εACA which prevents milk protease contamination of the precipitated fibrinogen.

CLAIMS

1. A method for the part purification of a desired protein from milk, the method comprising the transfer of protease enzyme which is present in the milk, into the whey phase with the removal or partition of the desired protein into another phase of the milk.

2. A method as claimed in claim 1, wherein the protease enzyme is plasmin and/or plasminogen.

3. A method as claimed in claim 1 or claim 2, wherein the removal or partition of the desired protein is by precipitation.

4. A method as claimed in any one of claims 1 to 3, wherein the transfer of the protease enzyme into the whey phase of the milk is by the presence of lysine or a lysine analogue

5. A method for the part purification of a desired protein from milk, the method comprising precipitation of the desired protein in the presence of lysine or a lysine analogue.

6. A method, as claimed in any one of claims 1 to 5, wherein the desired protein is a transgenic protein.

7. A method, as claimed in any one of claims 1 to 6, wherein the protein is fibrinogen, collagen, fibronectin, Factor VIII or alpha-2-macroglobulin.

8. A method, as claimed in any one of claims 1 to 7, wherein the milk is whole milk, skimmed milk, or milk fraction.

9. A method, as claimed in any one of claims 1 to 8, wherein the milk is derived from a sheep, cow, goat, rabbit, camel, water buffalo, pig or horse.

10. A method, as claimed in any one of claims 6 to 9, wherein the transgenic protein is bovine or human derived.

11. A method as claimed in any one of claims 1 to 10, wherein the part purified protein is further purified by repeating the purification method.

12. A transgenic protein, obtainable from milk, at least partly purified, having improved stability, in particular in respect of proteolysis.

13. A transgenic protein, as claimed in claim 12, which is fibrinogen, fibronectin, collagen, Factor VIII, or alpha-2-macroglobulin.

14. A protein obtainable by a method as claimed in any one of claims 1 to 11.

15. The use of lysine or a lysine analogue in the purification of a transgenic protein from milk.

16. The use, as claimed in claim 15, in combination with precipitation of the transgenic protein.

17. A fibrin adhesive or sealent containing fibrinogen as claimed in any one of claims 12 to 14.

18. A fibrin adhesive or sealent as claimed in claim 17, which contains thrombin, Ca^+ and Factor XIIIa and/or Factor XIIIa.

19. A kit for a fibrin adhesive or sealent, comprising fibrinogen as claimed in any one of claims 12 to 14 together with Factor XIII and/or Factor XIIIa as one component and thrombin and Ca^+ as a second component.

20. A method for producing a fibrin adhesive or sealent as claimed in claim 17 or claim 18, comprising admixing fibrinogen with thrombin.

21. Fibrinogen, as claimed in any one of claims 12 to 14, for use in medicine.

22. A fibrin adhesive or sealent, as claimed in any one of claims 17 to 19, for use in medicine.

23. A method of surgery or therapy comprising placing a fibrin adhesive or sealent, as claimed in any one of claims 17 or 18, on or within an animal or a body part of an animal.

24. A method as claimed in claim 23, wherein the animal is a human.

25. The use of fibrinogen, as claimed in any one of claims 12 to 14, in the manufacture of a fibrin adhesive or sealent.

26. A method for the part purification of a protein from milk, substantially as hereinbefore described with reference to the example.

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